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SUSCEPTIBILITY

Antifungal Susceptibility of 182 *Fusarium* Species Isolates from 20 European Centers: Comparison between EUCAST and Gradient Concentration Strip Methods

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ABSTRACT We determined the susceptibility of 182 *Fusarium* species isolates to five antifungal drugs (amphotericin B, voriconazole, posaconazole, isavuconazole, and terbinafine) by the EUCAST method. Based on the latest taxonomic insights, isolates collected from 20 European centers were distributed into seven complexes and 27 species. The susceptibility was variable, depending on the species. Comparison with the gradient concentration strip method, which was used for 77 isolates, showed essential agreement values for voriconazole, posaconazole, isavuconazole, and amphotericin B of 17%, 91%, 83%, and 70%, respectively.

KEYWORDS *Fusarium*, antifungal susceptibility, comparison, EUCAST, gradient concentration strips, amphotericin B, voriconazole, posaconazole, isavuconazole, terbinafine, Etest

The environmental fungi *Fusarium* spp. are responsible for opportunistic infections in humans, animals, and plants (1–4). *Fusarium* spp. are known to be rather unsusceptible to antifungals, but differences in susceptibility profiles for different drugs have been reported, depending on the species (5). Currently, *Fusarium* spp. include over 300 species, grouped into

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23 phylogenetic species complexes (SCs) (6). Given the highly complex classification and rapidly evolving taxonomy, there is a need to update susceptibility determinations according to SCs and species as knowledge evolves and new species are described.

We determined the antifungal susceptibility of 182 *Fusarium* sp. isolates distributed into seven SCs and 27 different phylogenetic species. Isolates were collected from 20 European centers between 1 January 2018 and 31 December 2018 and sent to our institution for identification and antifungal susceptibility testing. Identification was based on a DNA phylogenetic tree-based approach (7). The MICs of amphotericin B, voriconazole, posaconazole, isavuconazole, and terbinafine were determined by using the EUCAST standardized methodology for all isolates and by using gradient concentration strip (GCS) methods (except for terbinafine) for 77 isolates belonging to the most frequent species, i.e., those represented by at least 8 isolates. For EUCAST methodology, drugs concentrations ranged from 0.03 to 16 mg/liter for all tested drugs except posaconazole, which ranged from 0.016 to 8 mg/liter. The MIC values were obtained by visual assessment of the turbidity after 48 h of growth at 37°C and were determined as the lowest concentration with complete inhibition of growth. *Candida parapsilosis* strain ATCC 22019 was used for internal quality control in each EUCAST microplate (even for terbinafine, although there is no defined target value). The GCS method was performed using MIC test strips (Liofilchem, Italy) for isavuconazole and Etest strips (bioMérieux, France) for amphotericin B, voriconazole, and posaconazole. Essential agreement (EA) between methods was considered to be achieved when the MIC values were within the range of 2 dilutions. Correlation between methods was assessed using the Pearson's correlation test.

The comprehensive set of results, showing all MIC values determined by the EUCAST and GCS methods for the different antifungal agents and for all analyzed isolates, is presented in Table S1 in the supplemental material. The main results are presented in Table 1. The majority of isolates were distributed within three SCs, namely, the *Fusarium oxysporum* SC (FOSC), the *Fusarium fujikuroi* SC (FFSC), and the *Fusarium solani* SC (FSSC), reflecting the epidemiology of human fusariosis (7, 8). As expected, *Fusarium* spp. were characterized by low *in vitro* susceptibility to antifungal drugs. However, significant variations in MIC values between the different SCs were observed. Furthermore, within a given SC, some heterogeneity in MIC values was observed, which seemed to be not related to the species identification (see Table S1).

As previously reported, low susceptibility of the FSSC appears to be the rule, regardless of the antifungal drug considered, including the most recently marketed azole isavuconazole (9). Interestingly, isolates of the FFSC were distinguished by greater susceptibility to terbinafine, compared to isolates from other complexes. Overall, amphotericin B was the drug with the lowest geometric mean MIC value (2.3 mg/liter).

EUCAST (readout at 48 h) and GCS (readout at 24, 48, or 72 h) methods showed rather good correlation overall, with the exception of voriconazole. At the 48-h readout, the EA values for voriconazole, posaconazole, isavuconazole, and amphotericin B were 17%, 91%, 83%, and 70%, respectively. However, some variations according to the drug and the incubation period for the GCS method were noticed. For posaconazole and isavuconazole, good correlation was achieved within the first 24 h of growth (rho = 0.58 [$P < 0.0001$] and rho = 0.64 [$P < 0.0001$], respectively); for amphotericin B, the best correlation was obtained after 72 h of growth (rho = 0.6 [$P < 0.0001$]). Lastly, for voriconazole, the correlation coefficients were lower, although statistically significant, whatever the growth period (rho = 0.3 [$P < 0.008$] after 48 h and rho = 0.5 [$P < 0.0001$] after 72 h).

Our study includes a very large number of molecularly identified isolates of *Fusarium* spp. collected in 20 European centers. To the best of our knowledge, these results represent the largest series of *Fusarium* sp. isolates for which a reference method for antifungal susceptibility testing has been used. This work presents a number of pitfalls and limitations. In particular, the interpretation is challenged by the fact that important data, such as categorical endpoints or clinical breakpoints, are still lacking for *Fusarium* spp. Espinel-Ingroff et al. proposed epidemiological cutoff values (ECVs) based on CLSI methods for two SCs plus the species *Fusarium verticilloides* (10), but there is still a long way to go. In addition, some species were represented by a small number of isolates, making it impossible to produce reliable aggregate data. Finally,

TABLE 1 MIC values determined by EUCAST and GCS methods for 182 clinical isolates of *Fusarium* spp.

Method, SC, and drug	MIC range (mg/liter)	MIC geometric mean (mg/liter)	No. of isolates with MIC of[a]:									EA (%) vs EUCAST method[b]
			0.125 mg/liter	0.25 mg/liter	0.5 mg/liter	1 mg/liter	2 mg/liter	4 mg/liter	8 mg/liter	16 mg/liter[c]	>16 mg/liter	
EUCAST method[d]												
Total (182 isolates)												
TBF	1 to >16	13.8	0	0	0	3	17	38	12	0	112	
VCZ	2 to >16	14.3	0	0	0	0	6	22	43	35	76	
PSZ	0.5 to >8	13.1	0	0	3	5	6	0	0	168		
ICZ	2 to >16	24.6	0	0	0	0	2	3	12	28	137	
AMB	0.25 to >16	2.3	0	1	5	62	61	16	19	15	3	
FFSC (54 isolates)												
TBF	1 to >16	3.9	0	0	0	2	13	31	4	0	4	
VCZ	2 to >16	11.6	0	0	0	0	2	6	16	21	9	
PSZ	0.5 to >8	11.0	0	0	3	2	2	0	0	47		
ICZ	2 to >16	24.1	0	0	0	0	1	2	4	4	43	
AMB	0.5 to 16	2.6	0	0	2	17	16	4	7	8	0	
FOSC (65 isolates)												
TBF	1 to >16	20.9	0	0	0	1	1	5	8	0	50	
VCZ	2 to >16	11.0	0	0	0	0	2	14	22	6	21	
PSZ	1 to >8	13.9	0	0	0	1	3	0	0	61		
ICZ	2 to >16	20.9	0	0	0	0	1	0	7	22	35	
AMB	0.5 to >16	2.2	0	0	1	21	27	5	6	3	2	
FSSC (53 isolates)												
TBF	>16	32.0	0	0	0	0	0	0	0	0	53	
VCZ	8 to >16	28.4	0	0	0	0	0	0	1	7	45	
PSZ	>8	16.0	0	0	0	0	0	0	0	53		
ICZ	>16	32.0	0	0	0	0	0	0	0	0	53	
AMB	0.25 to >16	1.9	0	1	2	21	15	6	5	2	1	
GCS method[e]												
Total (77 isolates)												
VCZ	0.25 to >16	2.2	0	2	11	15	19	18	4	2	6	17
PSZ	0.125 to >8	10.8	1	0	7	0	0	1	0	68		91
ICZ	0.125 to >16	17.2	1	0	0	6	7	1	0	0	62	83
AMB	0.25 to >16	1.5	0	3	12	24	22	8	6	1	1	70
FFSC (18 isolates)												
VCZ	0.25 to 16	1.3	0	1	5	4	4	3	0	1	0	22
PSZ	0.125 to >8	3.8	1	0	6	0	0	0	0	11		72
ICZ	1 to >16	9.0	0	0	0	5	2	0	0	0	11	72
AMB	0.5 to 16	2.1	0	0	1	5	8	1	2	1	0	67
FOSC (20 isolates)												
VCZ	0.25 to 8	0.9	0	1	6	9	3	0	1	0	0	5
PSZ	0.5 to >8	12.6	0	0	1	0	0	1	0	18		90
ICZ	0.125 to >16	9.2	1	0	0	1	5	1	0	0	12	60
AMB	0.5 to >16	2.1	0	0	3	5	5	3	3	0	1	70

(Continued on next page)

TABLE 1 (Continued)

Method, SC, and drug	MIC range (mg/liter)	MIC geometric mean (mg/liter)	No. of isolates with MIC of[a]:									EA (%) vs EUCAST method[b]
			0.125 mg/liter	0.25 mg/liter	0.5 mg/liter	1 mg/liter	2 mg/liter	4 mg/liter	8 mg/liter	16 mg/liter[c]	>16 mg/liter	
FSSC (39 isolates)												
VCZ	1 to >16	4.5	0	0	0	2	12	15	3	1	6	21
PSZ	>8 to >8	16.0	0	0	0	0	0	0	0	39		100
ICZ	>16 to >16	32.0	0	0	0	0	0	0	0	0	39	100
AMB	0.25 to 8	1.1	0	3	8	14	9	4	1	0	0	72

[a]MIC values for the GCS method were obtained with a 48-h readout. TBF, terbinafine; AMB, amphotericin B; ICZ, isavuconazole; PSZ, posaconazole; VCZ, voriconazole.
[b]EA between methods was considered to be achieved when the MIC values were within ±2 dilutions.
[c]For posaconazole, concentrations ranged from 0.016 to 8 mg/liter; therefore, this column should be interpreted as ≥8 mg/liter.
[d]Total, FFSC, FOSC, FSSC, *Fusarium culmorum* SC, *Fusarium dimerum* SC, *Fusarium incarnatum* SC, and *Fusarium redolens* SC; FFSC, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium sacchari*, *Fusarium acutatum*, *Fusarium andiyazi*, and *Fusarium lactis*; FOSC, *Fusarium veterinarium*, *Fusarium nirenbergiae*, *Fusarium curvatum*, *Fusarium elaeidis*, *Fusarium gossypinum*, *Fusarium languescens*, and *Fusarium triseptatum*; FSSC, *Fusarium falciforme*, *Fusarium keratoplasticum*, *Fusarium petroliphilum*, FSSC new species 1, FSSC new species 2, *Fusarium lichenichola*, *Fusarium robiniae*, *F. solani* FSSC5, and *F. solani* FSSC9. FOSC, *Fusarium oxysporum* species complex; FFSC, *Fusarium fujikuroi* species complex; FSSC, *Fusarium solani* species complex.
[e]Total, FFSC, FOSC, and FSSC; FFSC, *F. verticillioides* and *F. proliferatum*; FOSC, *F. veterinarium* and *F. nirenbergiae*; FSSC, *F. falciforme*, *F. keratoplasticum*, *F. petroliphilum*, FSSC new species 1, and *F. solani* FSSC5.

whether clinical breakpoints will be available some day is uncertain, as a clear and definite relationship between MICs obtained *in vitro* and therapeutic efficacy during the course of fusariosis is doubtful (11, 12). Similarly, a recent study found no correlation between MIC values and mortality rates at day 90 (4) (although this point does not take into consideration the given treatment), while another report indicated that a patient with fusariosis due to an isolate with a high itraconazole MIC showed clinical improvement after treatment with this antifungal agent (13). In any case, beyond the potential clinical impact, the importance of conducting epidemiological surveys of *Fusarium* sp. susceptibility to antifungal agents has two additional justifications. The first is the rapid evolution of taxonomy and the fact that data generated at one time may become inaccurate soon thereafter. The second is the environmental nature of *Fusarium* spp., because of which they are prone to have their resistance profiles evolve according to their exposure to antifungal agents used in agriculture (especially demethylase inhibitors), as is the case with *Aspergillus fumigatus*.

In a previous work, EUCAST and GCS methods showed an EA above 85% but the study included only 20 *Fusarium* sp. isolates and as many different species (14). For us, the results of the EUCAST and GCS methods were correlated. However, similar to what we reported concerning *Aspergillus* section *Nigri* (15), we found consistently lower MIC values with the GCS method, compared with the EUCAST method, particularly for azole drugs. Consequently, the EA values were variable, being particularly low for voriconazole (<25%) and more suitable (70% to 91%) for the other azole drugs. As our work provides new data on the susceptibility of *Fusarium* spp. to antifungal agents, we hope that it can contribute in the future to the establishment of accurate ECVs for a wider range of species.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.03 MB.

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